

MECHANISMS OF ACTION FOR CONTROL OF SOILBORNE PATHOGENS BY HIGH NITROGEN-CONTAINING SOIL AMENDMENTS.

Mario Tenuta and **George Lazarovits***, Agriculture and Agri-food Canada, Southern Crop Protection and Food Research Centre, 1391 Sandford St., London, Ontario, Canada, N5V 4T3 (Lazarovitsg@em.agr.ca; phone 519-663-3099)

Our laboratory has tested various products from the rendering industry (meat and bone meal, feathermeal, and bloodmeal) as to their ability to reduce soilborne pathogens of crops. The products, when tested on a potato crop, were all highly effective in reducing the incidence of early dying syndrome (caused by *Verticillium dahliae*), common scab (caused by *Streptomyces scabies*), and lesion, pin, and rootknot nematodes (various genera). The amendments were as effective as chemical control measures suggesting a possible use as alternatives to chemical fumigants such as methyl bromide.

The utilization of rendering products as fumigant alternatives, however, is limited by; a) a requirement for high application rates making the practice uneconomical and b) inconsistent control of pathogens from site to site. In order to determine if these problems can be overcome, we are trying to determine the mechanisms by which rendering products kill soil pathogens. Such information is deemed vital for formulating products at rates useful to growers. Discovery of what soil properties determine the efficacy of a product is critical for recommendations as to where a rendering product may be utilized with success. For instance, adding microbial inoculum to a formulation may promote the release of bioactive compounds and may lead to reduced rates required to kill plant pathogens. In this way formulations can be tailor-made to the needs of a specific site or crop.

Mechanism Studies

The role of ammonia accumulation in soil: We have shown that in closed containers all resting structures of *V. dahliae* microsclerotia (MS) die within 7 days when in the presence of soils amended with high nitrogen-containing organic amendments, even though they are suspended in the head space above the soils. Thus, rapid death of MS results from the release of toxic volatile gases from the organic product. When the soil and amendments are sterilized, MS are unaffected indicating that the gases are derived from microbial degradation of the organic material. In tests using a diverse range of soil types from various locations in Canada and the world, we added various rates of meat and bone meal (MBM) amendment ranging from 0, 0.5, 1.0, and 2.0% (w w⁻¹). In a sand soil (soil A) we found that all MS buried in the soil were killed within 4 days after incorporation of 2% MBM (Fig. 1). In this soil, in excess of 55 mg N kg⁻¹ of ammonia (NH₃) had accumulated by day 4 (Fig. 1). In this soil a 1% rate of MBM did not result in very high concentrations of ammonia yet the MS all died, but it now took over 14 days (Fig. 1). Lower concentrations of MBM had no effect on MS survival. In soil B, a loam soil, even the 2% rate of MBM failed to control *V. dahliae* MS within one week

after addition (Fig. 1). In this soil there was no evidence of any measurable levels of ammonia accumulation (Fig. 1, soil B). In soil B, however, instead of ammonia,

we found that significant quantities of nitrate accumulated (data not shown). The toxicity of ammonia to MS was further confirmed by exposing them to various concentrations of ammonia in agar media (Fig. 2).

Comparison of ammonia accumulation in 12 different soils amended to 2% (w w⁻¹) MBM revealed that soil organic carbon was a critical factor for how much ammonia accumulated in soil after incorporation of a high nitrogen amendment (Fig. 3). The relationship between organic carbon and the peak ammonia levels reached in the various soils after addition of 2% by weight of MBM is shown in (Fig. 3). In soils with an organic carbon content of greater than 1.7%, even application of 2% (w w⁻¹) MBM would not result in ammonia accumulation and *V. dahliae* control. The role of organic matter in regulation of ammonia release was further clarified by addition of peat (from a marsh) at 2% or 4% rate (w w⁻¹) to a sandy soil. Adding 2% peat to the sandy soil (soil A) reduced ammonia accumulation and soil pH increase such that 2% MBM addition did not reduce MS viability, whereas in untreated soil, 100% eradication was achieved (Fig. 4).

We tested if higher rates than 2% MBM resulted in ammonia release in the loam soil. Adding 4% (w w⁻¹) MBM to this was found to result in significant ammonia accumulation and MS eradication (Fig. 5). Thus, it appeared that ammonia production can be achieved in any soil provided that soil organic carbon content is accounted for by adjusting amendment rates accordingly. By calculating this factor for each soil, the application rates of rendering products can be adjusted to provide a more consistent control of soilborne pathogens from site to site.

The role of nitrification in soil: In many soils amended with rates of MBM that did not result in ammonia accumulation, MS were found to be killed within 2-5 weeks after incorporation. In such soils we observed a consistent lowering of soil pH. The transformation of ammonium to nitrate in soil is the major process reducing soil pH following amendment with products high in organic nitrogen such as those of the rendering industry. We therefore tested what affect adding an inhibitor of nitrification, dicyandiamide (DCD), would have on the survival of *V. dahliae* MS in soil amended with MBM. DCD targets specifically autotrophic nitrifying bacteria preventing the crucial first step in the oxidation of ammonium by the enzyme ammonia monooxygenase. Without the addition of DCD, a reduction in soil pH and accumulation of the nitrification products nitrite (NO₂⁻) and nitrate (NO₃⁻) were associated with death of *V. dahliae* 2 weeks following amendment to 1% MBM (Fig. 6). Inclusion of DCD with MBM to soil resulted in a soil pH poised above 8 throughout the study, the absence of nitrification products, and continued survival of *V. dahliae* (Fig. 6). From this particular study, there is strong evidence that the 2% MBM addition resulted in ammonia toxicity to *V. dahliae* but at 1% MBM, death was associated with nitrification. Particularly since DCD is a specific inhibitor of autotrophic nitrifying bacteria, these organisms are implicated to be involved. Studies are in progress to confirm if nitrification is directly involved in the death of *V. dahliae*, to evaluate if the numbers and activity of nitrifiers can predict efficacy of

Conclusions: Application of high nitrogen-containing organic amendments have been shown by us and others to effectively control a wide spectrum of plant pathogens. There are, however, limitations to the use of these products. At the rates required for many soils the cost of product would be prohibitive. In soils with less than 1% organic matter, however, our data predicts that rates of 5 metric tons per hectare (ca 5 tons per acre) would be sufficient to control most plant pathogens. This calculation is based on broadcast application and incorporation to a depth of 15 cm (6 inches). With more judicious application to rows only, the rate could be further reduced. The current cost of MBM is about \$175 per metric ton. At this cost and rate, MBM would be cost competitive with methyl bromide. In addition, growers could significantly reduce their fertilizer costs as MBM at the rates suggested provides all the nitrogen a crop would require. We have also seen that disease protection can extend for several years and that yearly applications may not be required. The long term use of organic products has many other beneficial effects on soil microbiology and physical composition. There is also some promise for use of such amendments for soils with organic carbon contents higher than 1%. Here, lower rates of organic product may work by yet an unidentified mechanism that is associated with nitrate formation and a reduction in soil pH. Despite the multiple reports of the effectiveness of organic products for disease control, the research effort toward making these products work effectively under field conditions remains minimal. The indications are, however, that with greater understanding of the mechanisms by which organic amendments work, they can play a role as replacements for methyl bromide.

Figure 1. Viability of microsclerotia of *Verticillium dahliae* and ammonia accumulation in two soils (A. Beauseart; sand; B. Thorndale; loam) following addition of meat and bone meal (MBM).

Figure 2. Viability of microsclerotia of *Verticillium dahliae* grown at various concentrations of ammonia in Soil-Pectate-Tergitol agar.

Figure 3. The dependency of peak ammonia accumulation to soil organic C content in 12 soils following amendment with 2% MBM ($w w^{-1}$).

0% Peat

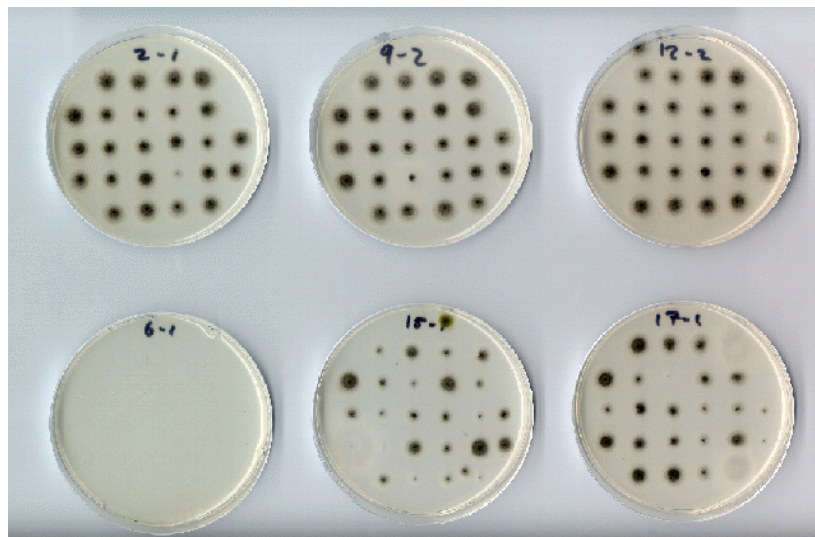


Figure 4. Viability of *Verticillium dahliae* microsclerotia 9 days following addition of Holland Marsh Peat and meat and bone meal (MBM) to a Beauseart sand soil.

Figure 5. Viability of *Verticillium dahliae* microsclerotia, changes in soil pH, and accumulation of ammonia, nitrite, and nitrate following incorporation of meat and bone meal to a Thorndale loam soil.

Figure 6. Viability of *Verticillium dahliae* microsclerotia, and accumulation of ammonia, nitrite, and nitrate in soils following addition of meat and bone meal (MBM) with (+DCD) or without (-DCD) the nitrification inhibitor dicyandiamide.

